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# SOURCES OF ERROR IN DIRECT MICROSCOPIC METHODS FOR ESTIMATION OF FUNGAL BIOMASS IN SOIL*

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**Summary**—Sources of error in direct microscopic measurement of fungal hyphae in soil were examined and fungal biomass estimates and associated variability obtained by the direct counting method and the ergosterol technique were compared. Nested random effects ANOVA indicated that the major source of variance in the direct microscopic counting method were the people examining the prepared microscope slides, accounting for 83% of the total variance. Sampling variability accounted for approximately 14% of the total variance. Fungal biomass values calculated from soil ergosterol concentrations were close to the range of values derived from hyphal length estimates but coefficients of variation were much lower for soil ergosterol determinations (6–13%) than for hyphal length estimates (16–32%). For one soil sample, we compared total hyphal length and fungal biomass estimates from our lab to those of another lab. Values obtained by the other laboratory were outside the range of values and 95% confidence intervals reached in our lab. Comparison of fungal hyphal length estimates from undisturbed prairie soil and an adjacent cultivated soil indicated that the undisturbed soil contained more than twice as much fungal biomass. Results of our study indicate: (1) extreme caution must be used when comparing hyphal length and fungal biomass estimates made by different laboratories using the direct counting method; and (2) soil ergosterol determinations can provide information on fungal biomass that may be useful in comparing direct count estimates by different labs.

## INTRODUCTION

The importance of fungi to soil properties and processes is well recognized, yet precise quantification of these organisms is problematic (Parkinson, 1982; Frankland, 1990; Frankland *et al.*, 1990). Accurate and reliable data for fungal biomass are essential to quantitatively assess their role in such functions as decomposition, nutrient cycling, food webs and soil aggregation (Christensen, 1989; Newell, 1992).

A number of methods have been described to estimate fungal biomass in soil including the use of specific biochemical components of fungal cells (chitin, ergosterol, phospholipid-fatty acids), measurement of metabolic activity (selective respiratory inhibition), viable counts and direct microscopic counting. There are, however, serious potential sources of error in all of these techniques (Frankland *et al.*, 1990; Parkinson and Coleman, 1991). Problems with direct microscopic counting include hyphae hidden in soil aggregates, calculation of biomass from counts, variability associated with results and observer subjectivity (Söderström, 1979; Bååth and Söderström, 1979). Nevertheless, this technique has less serious drawbacks than many of these others and remains the most widely accepted and commonly used method to estimate fungal biomass in soil. For this reason, it is important to better understand the limitations of the direct counting method and to work towards improvement of this technique.

Evaluations and comparisons of the various ways to perform this assay (e.g. agar film and membrane filter techniques, various stains, counting procedures) are described in detail in the literature (Bååth and Söderström, 1980; Ingham and Klein, 1984; West, 1988). Lodge and Ingham (1991) investigated sources of variation among a number of adaptations of the agar film technique but did not examine the influence of different observers. Because observer subjectivity is a major criticism of direct microscopic counting (Domsch *et al.*, 1979; Morgan *et al.*, 1991), it is essential to evaluate the influence of this factor on results obtained by this method.

The primary goal of our study was to determine the major sources of error in direct microscopic counting of hyphae to estimate fungal biomass in soil and recommend procedures to minimize those errors. Specifically, using soil from a relatively undisturbed native prairie site and an adjacent cultivated soil, we: (1) examined the sources of error associated with the direct counting method including the influence of observers; (2) compared results obtained by two

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different labs examining the same soil sample; and (3) compared fungal biomass estimates and associated variability obtained by the direct microscopic counting method and the ergosterol method.

## METHODS

Soil samples were collected from an undisturbed prairie site at the Doolittle Native Prairie Preserve in Story County, IA on 16 May 1993; samples of cultivated soil were obtained on the same date from a corn–soybean field within 25 m of the undisturbed site. The soil at both sites was a Kossuth silty clay loam (Typic Haplaquoll). Our sampling procedure is illustrated in Fig. 1. Three samples of approximately 100 g (5–15 cm depth) were collected from within a 10 m square area at each of the two sites (undisturbed and cultivated). Samples were returned to the lab, sieved (5 mm sieve), and each was divided into three subsamples. Three 1 g aliquots of soil were taken from each seived subsample to prepare slides for direct microscopic examination of fungal hyphae. All slides were made within 36 h of soil collection. Each slide was examined by 4 different observers within 45 days of preparation (examination of a number of slides when first prepared and 60 days after preparation indicated that amounts of fungal hyphae on prepared slides did not increase or decrease in that amount of time). Thus, the components of variability analyzed were those associated with distribution of fungal hyphae among samples, hyphal distribution among subsamples, slide preparation and observer variability. Finally, 5 g of soil was removed from each subsample for analysis of ergosterol content and soil moisture content was determined gravimetrically on a dry weight basis for all subsamples.

Fungal hyphae in soil were examined directly using the membrane filter method (Hanssen *et al.*, 1974) with calcofluor M2R white (Sigma, St Louis, MO) as stain and the gridline intersect method (Olson, 1950) for measuring hyphal lengths. For each slide made, 1 g soil (wet wt) was dispersed in 500 ml nanopure filtered water in a Waring blender at highest speed for 1.0 min. 1 ml of the suspension was immediately passed through a 25 mm dia 0.4 $\mu$m mesh polycarbonate membrane filter (using a widemouth pipette tip). The material remaining on the filter was then stained for 15–20 s with 1 ml of a 2.3 $\mu g\,ml^{-1}$ aqueous solution of calcofluor white dispensed with a syringe equipped with a 0.2 $\mu$m filter. Stain was removed by vacuum followed by rinsing with nanopure filtered water. The stained filter was placed on a glass microscope slide, allowed to air dry for a few seconds to remove excess moisture, mounted with 2 drops of immersion oil and covered with a glass cover slip. For each soil (undisturbed vs cultivated), 27 slides were made (3 samples × 3 subsamples × 3 slides).

Slides were examined with a Nikon Microphot-SA epifluorescent microscope equipped with a high intensity Hg light source and a filter cube (Nikon UV-1A). Observations were made using a dry 40 × objective, 10 × eyepieces and 1.5 × light path magnifier (total magnification—600 ×); 25 randomly-chosen fields of

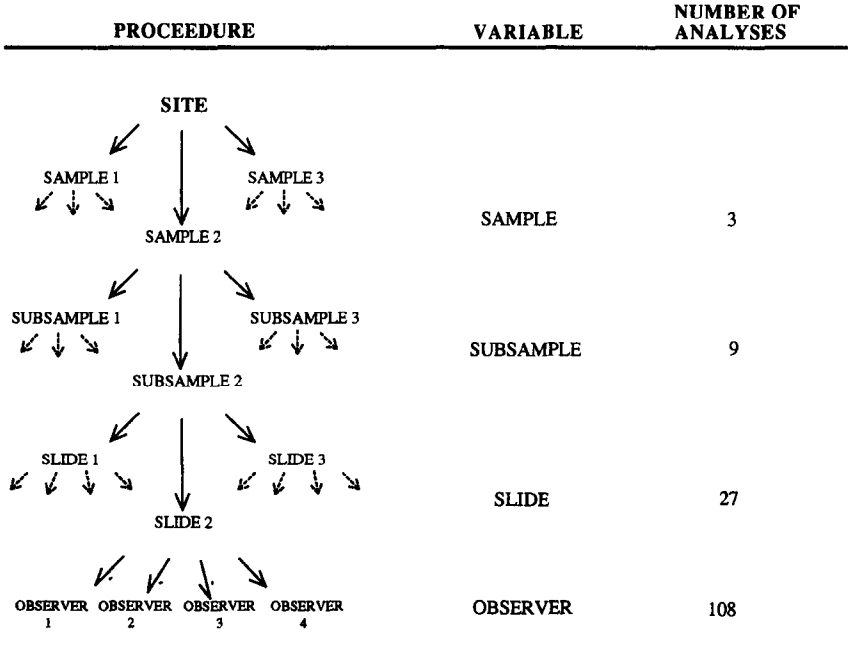


Fig. 1. Diagrammatic representation of the experimental design showing the sampling procedure, names of variables and numbers of analyses.

Table 1. Nested random effects ANOVA for direct microscopic counts of fungal hyphae in undisturbed prairie soil

| Variance source | *df* | Sum of squares | Variance component | Percent of total | *F*-value | *P* |
|---|---|---|---|---|---|---|
| Total | 107 | 903.428 | 10.476 | 100 | | |
| Sample | 2 | 119.423 | 1.514 | 14.45 | 11.495 | 0.0088 |
| Subsample | 6 | 31.165 | 0.224 | 2.13 | 2.073 | 0.1077 |
| Slide | 18 | 45.087 | −1.558 | 0.00 | 0.287 | 0.9979 |
| Observer | 81 | 707.752 | 8.737 | 83.40 | | |
| Error | 0 | | | | | |

view were counted on each slide. All slides were made by the same investigator and each slide was counted by 4 different observers. Observers were trained together at the microscope by inspecting prepared direct microscopic count slides and discussing observations with the trainer. Observers were instructed in the differentiation of fungal hyphae from actinomycete filaments, cellulose fibers and other non-fungal filaments on slides, as well as counting hyphal grid-line intercepts and determination of hyphal diameters. As part of the training, all observers counted hyphal intercepts in several of the same microscope fields and then compared and discussed results in an attempt to standardize observations of all 4 observers. Actual measurements of fungal hyphae for data reported in this study were made independently by each observer at their own pace.

Estimates of fungal biomass were calculated from hyphal lengths using two methods, one in which an average value for hyphal dia of 3.0 $\mu$m (based on observation and measurement of hyphae from the soil samples) was used to calculate biovolume, and one in which hyphae were classified by diameter into size groups (small, $<2.0\,\mu$m dia; medium, 2.0–6.0 $\mu$m dia; large, $>8.00\,\mu$m dia; for calculations, average dia values of 1.5, 3.0 and 6.0 $\mu$m, respectively, were used) during microscopic counting. Calculations were made using the equation given by Paul and Clark (1989):

$$B_f = \pi r^2 L\, e\, S_c,$$

where $B_f$ is fungal biomass, $L$ is hyphal length (cm g soil$^{-1}$), $e$ is hyphal density (1.3 g cm$^{-3}$), and $S_c$ is solids content (0.3).

Estimates of fungal biomass on soil ergosterol measurements were calculated using the only published value for ergosterol content of fungi grown in soil of 0.16 $\mu$g ergosterol cm$^{-2}$ fungi (West *et al.*, 1987). We converted this value based on fungal surface area to one based on biomass by calculating both the surface area and biomass of estimated lengths of fungal hyphae, assuming a mean hyphal dia of 3.0 $\mu$m, to obtain an average value of 5.48 $\mu$g ergosterol mg dry fungal biomass$^{-1}$.

Ergosterol was extracted from soil using the following method. Soil samples of 5 g were placed in 16 × 125 mm centrifuge tubes with 15 ml cold methanol and 5 ml 4% potassium hydroxide (in 95% ethanol). Each tube was vortex mixed for 10 s and sonicated (2 min at medium power, 10% duty cycle, extended probe; sonicator manufactured by Sonics and Materials, Danbury, CT) before being placed in an 85°C water bath for 30 min. Tubes were then cooled to room temperature and 5 ml of water was added to each. The soil solution was vacuum filtered through Whatman No. 4 filter paper followed by washing with 5 ml methanol. Hexane (5 ml) was then added to each tube and the tubes were inverted by hand 20 times to transfer the ergosterol from the methanol phase to the hexane phase. The hexane layer (top) was removed and saved. The hexane extraction process was repeated 3 times and hexane from each sample combined. Tubes containing the hexane phase were dried in an automatic evaporation workstation (Turbovap, Zymark Corp., Hopkinton, MA) under $N_2$. When the tubes were dry, 5 ml of methanol was added to each tube followed by vortex mixing and dried down again to 1.0–1.5 ml vol. Samples were then removed from the tubes with a syringe and expelled through a 0.2 $\mu$m syringe filter into amber HPLC vials. Ergosterol in samples was quantified by HPLC analysis using a Hewlett–Packard 1090A HPLC equipped with a diode array detector. A mobile phase of methanol–water (95:5 v/v) was used with a ramped flow rate of 0.5–2 ml min$^{-1}$ with a LiChrospher 100 RP-18 column and oven temperature of 40°C. Ergosterol retention time was ca. 5.4 min.

A second comparison of observer estimates of total fungal hyphal length in the undisturbed soil was conducted on a sample collected on 1 October 1993. In this experiment each observer made her or his own slide for examination from a 1 g soil subsample removed directly from the sample bag. Approximately half of the remaining sample was then shipped overnight express to Microbial Biomass Service (Corvallis, OR) for an independent analysis of total fungal hyphal length and biomass.

Nested random effects analysis of variance (ANOVA) and two-factor ANOVA were used to determine sources of variance in this study (SAS Institute, 1988) and paired samples *t*-tests were used to compare population means (SYSTAT, 1992).

## RESULTS

Nested random effects ANOVA (Table 1) indicated that the major source of variance in our procedure for estimating fungal hyphal length in soil was the ob-

Table 2. ANOVA for direct microscopic counts of fungal hyphae in soil

| Variance source | *df* | Sum of squares | *F*-value | *P* |
|---|---|---|---|---|
| Observer | 3 | 362.086 | 60.89 | 0.0001 |
| Sample | 2 | 119.423 | 30.12 | 0.0001 |
| Observer × Sample | 6 | 172.273 | 14.49 | 0.0001 |

servers which accounted for over 83% of the total variance. Sampling variability accounted for approximately 14% of the total variability. Variance components associated with subsamples and replicate slides were low and not significant. Single-factor ANOVA (Table 2) showed that the variance due to observers and samples was highly significant as was the interaction between these independent variables.

The estimates of fungal hyphal length in undisturbed prairie soil by each of the 4 observers given in Fig. 2 are based on the mean of the three samples collected at the site as determined by each observer. Values ranged from 684.2 to 1254.1 m g soil$^{-1}$, but 3 of the 4 estimates were between 684.2–776.8 m g soil$^{-1}$. Of the three observers that categorized fungal hyphae into size classes, observers 3 and 4 placed the majority of hyphae in the medium (2.0–6.0 $\mu$m dia) class, while observer 2 classified most of the hyphae as small (<2.0 $\mu$m dia). The estimated amount of large hyphae (>6.0 $\mu$m dia) was similar among the 3 observers. Coefficients of variation for estimates of the length of hyphae in the undisturbed soil by individual observers based on values from three samples ranged from 21.8 to 45.9%.

Determinations of hyphal lengths in undisturbed prairie soil vs adjacent cultivated soil by 3 observers

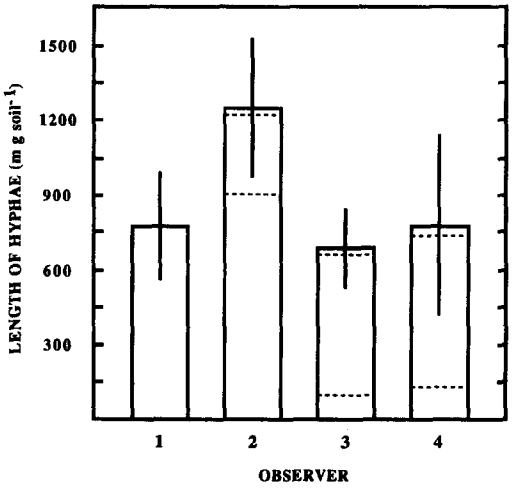


Fig. 2. Estimates of hyphal lengths in undisturbed prairie soil by 4 observers. Values given are the mean of 3 samples as determined by each observer. Vertical bars indicate ±1 SD. Dotted lines indicate the amount of hyphae in each size class: small (<2.0 $\mu$m dia) under the lowest dotted line; medium (2.0–6.0 $\mu$m dia) between the dotted lines; large (>6.0 $\mu$m dia) above the highest dotted line.

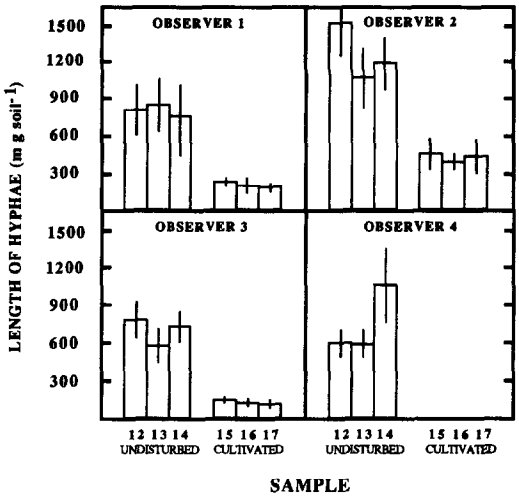


Fig. 3. Estimates of hyphal lengths in samples of undisturbed prairie and cultivated soils by 4 observers. Values given are the mean of 3 subsamples as determined by each observer. Vertical bars indicate ± 1 SD. Observer 4 did not estimate the length of hyphae in cultivated soil.

are given in Fig. 3. Values shown are means for each sample (based on 3 subsamples) as determined by each observer for each site. Differences in the fungal hyphal length values obtained by the observers for each sample are evident in Fig. 3; consistent values for samples within a site were reached by observers 1 and 3 but observer 4 found that sample 14 contained almost twice as much fungal hyphae as samples 12 and 13 from the same site (it is interesting to note that observer 4 counted prepared slides from sample 14 1 month after counting slides from samples 12 and 13; all slides were prepared at the same time). Although the 3 observers obtained different estimates of hyphal lengths in the 2 soils, all 3 found that undisturbed soil contained more than twice as much hyphae as cultivated soil.

Total fungal biomass mean values calculated from estimates of hyphal length for subsamples of soil from the undisturbed prairie site ranged from 1.65 to 4.16 mg g soil$^{-1}$ (Table 3). Biomass values calculated from hyphal length estimates using one average hyphal dia (3.0 $\mu$m) were different than those calculated using three size classes for hyphal dia (Table 3). This was especially true if a large proportion of hyphae was placed in the small (<2.0 $\mu$m dia) or large (>6.0 $\mu$m dia) categories. For example, for observer 2, who consistently classified a majority of hyphae as small, biomass values calculated by the two methods were quite different. In addition, there was a good deal of disparity among observers in classification of hyphae according to size.

Fungal biomass values calculated from ergosterol concentrations in soil were always close to the range of values derived from hyphal length estimates (Table 3). However, coefficients of variation based on values from 3 subsamples were much lower for soil

Table 3. Hyphal length estimates, calculated fungal biomass values and soil ergosterol determinations for the 3 soil samples from the undisturbed prairie site; biomass values were calculated using both an average value for hyphal dia (3.0 μm) and 3 hyphal dia size classes (see Methods)

| Sample | Ergosterol content (μg g soil$^{-1}$) | Observer | Hyphal lengths (m g soil$^{-1}$) | | | | Fungal biomass (mg g soil$^{-1}$) from: Hyphal lengths | | Ergosterol |
|---|---|---|---|---|---|---|---|---|---|
| | | | Small | Medium | Large | Total | Av. dia | Size classes | |
| 1 | 23.33 ± 2.16 | 1 | ND | ND | ND | 777.1 ± 199.7 | 2.14 ± 0.55 | ND | 4.26 ± 0.39 |
| | | 2 | 1186.8 ± 170.3 | 295.7 ± 186.9 | 26.9 ± 29.4 | 1512.0 ± 276.5 | 4.16 ± 0.76 | 2.16 | |
| | | 3 | 111.4 ± 41.0 | 609.4 ± 99.9 | 57.6 ± 51.2 | 778.4 ± 142.1 | 2.14 ± 0.39 | 2.89 | |
| | | 4 | 88.3 ± 235.8 | 462.2 ± 88.3 | 46.1 ± 37.1 | 600.5 ± 101.1 | 1.65 ± 0.28 | 2.23 | |
| 2 | 12.08 ± 0.67 | 1 | ND | ND | ND | 816.0 ± 208.5 | 2.25 ± 0.57 | ND | 2.20 ± 0.12 |
| | | 2 | 676.2 ± 200.1 | 356.8 ± 282.1 | 31.3 ± 41.0 | 1065.5 ± 236.3 | 2.94 ± 0.65 | 2.06 | |
| | | 3 | 88.0 ± 38.6 | 477.3 ± 110.9 | 13.3 ± 13.3 | 578.6 ± 132.6 | 1.59 ± 0.37 | 1.64 | |
| | | 4 | 125.4 ± 26.5 | 432.7 ± 95.2 | 37.4 ± 27.7 | 595.5 ± 98.8 | 1.64 ± 0.27 | 2.01 | |
| 3 | 9.46 ± 0.95 | 1 | ND | ND | ND | 728.4 ± 236.7 | 2.01 ± 0.65 | ND | 1.73 ± 0.17 |
| | | 2 | 846.8 ± 224.9 | 328.2 ± 186.1 | 16.1 ± 18.3 | 1185.7 ± 208.7 | 3.27 ± 0.58 | 1.80 | |
| | | 3 | 90.4 ± 23.7 | 613.3 ± 120.5 | 11.8 ± 18.3 | 715.5 ± 113.0 | 1.97 ± 0.31 | 1.98 | |
| | | 4 | 152.8 ± 31.2 | 985.6 ± 170.0 | 45.2 ± 40.9 | 1064.1 ± 309.9 | 2.93 ± 0.85 | 3.72 | |

ND = not determined.

ergosterol determinations (6–13%) than for hyphal lengths (16–32%).

Estimates of total hyphal length and fungal biomass from the undisturbed prairie soil sample by 5 observers (including an analysis by a different laboratory) ranged from 316.3 to 932.8 mg g soil$^{-1}$ and 0.64 to 2.57 mg g soil$^{-1}$, respectively (Table 4). Also shown in Table 4 are the mean and 95% confidence intervals for hyphal length and biomass calculated from the 4 determinations made in our lab. Values obtained by the independent laboratory were outside the range of values and 95% confidence intervals reached by our lab.

## DISCUSSION

Estimates of hyphal length in the undisturbed soil by the 4 observers in our lab were within the same order of magnitude but the high estimate (1254.1 ± 276.8 m g soil$^{-1}$) was almost double that of the lowest estimate (684.2 ± 149.6 m g soil$^{-1}$). There was no statistical difference among the values obtained by observers 1, 3 and 4, but the high estimate by observer 2 was significantly different from all others (paired samples $t$-tests, $df = 27$, $P < 0.0001$). Observer 2 also categorized hyphae by size differently than did observers 3 and 4, placing most hyphae in the small diameter class.

Despite the disparities among estimates of hyphal lengths, the 3 observers that compared undisturbed prairie soil to cultivated soil all found the former to have substantially more fungal hyphae. Ratios of fungal hyphae in undisturbed to cultivated soil obtained by the observers ranged from 2.76:1 to 5.29:1 The ratio of ergosterol content of undisturbed t cultivated soil was 2.53:1.

Table 4. Estimates of total fungal hyphal length and fungal biomass in a soil sample by 5 observers

| Observer | Hyphal length (m g soil$^{-1}$) | Calculated biomass (mg g soil$^{-1}$) |
|---|---|---|
| 1 | 811.2 | 2.23 |
| 2 | 932.8 | 2.57 |
| 3 | 675.4 | 1.86 |
| 4 | 586.1 | 1.61 |
| 97.5% confidence limits for observers 1–4 | 508–994 | 1.40–2.74 |
| Independent Lab | 316.3 | 0.64 |

The similarities and differences in the estimates should be viewed in the light of the fact that all 4 observers were examining the same set of slides on the same microscope and had prepared for and discussed the counting procedure before initiating the counts. Nested random effects ANOVA (Table 1) and single-factor ANOVA (Table 2) confirm that observer variability was the major source of error and was statistically highly significant. The interaction between observers and samples (Table 2) indicates that the length of fungal hyphae estimated for a particular sample was strongly influenced by who was doing the microscopic examination. The case of observer 4's counts of the undisturbed site (Fig. 3) suggests that the observer became "uncalibrated", i.e. after going for 1 month without counting slides, observer 4 interpreted what was seen on slides from sample 14 differently than samples 12 and 13.

A significant amount of variance was also attributed to the samples suggesting that the amount of fungal hyphae in each of the sampling locations was different. Little variance was associated with subsamples and slides, as was also reported by Lodge and Ingham (1991). In the same paper, Lodge and Ingham report a coefficient of variation among 3 litter samples collected on the same date of 28%, which falls within the range of values obtained for 3 samples by the observers in our study (22, 22, 28 and 46%). These results indicate that to minimize error in estimating the amount of fungal hyphae in soil, a large number of samples should be collected from the area of interest and a small number of slides be made directly from the samples; subsampling is not needed. The low amount of error associated with slides demonstrates that observation of 25 fields per slide is

a sufficient number and may be more than necessary. All counts of fungal hyphae on slides should be conducted by the same person or great effort should be made to minimize observer variability. The lack of a method to calibrate observer counts is a major problem in determining the accuracy of the direct microscopic counting method.

Fungal biomass values calculated by 3 different methods all yielded approximately the same range of estimates for the undisturbed prairie soil (1.59–4.26 mg g soil$^{-1}$). Results did not provide evidence that either of the methods for calculating fungal biomass from direct counts (average diameter vs size classes) was superior to the other. In theory, categorizing hyphae into size classes should give a more accurate conversion to biomass but in practice classification may be an additional source of error. Calculation of fungal biomass from soil ergosterol content is not a widely used method partly because the ergosterol concentration in fungal tissue in soil has not been sufficiently studied and conversion factors have not been adequately worked out. However, fungal biomass estimates based on soil ergosterol content were similar to those based on hyphal length but the variance associated with ergosterol determination was much lower than that of hyphal length estimates.

Ergosterol is found primarily in living tissue of eumycotic fungi (Weete and Weber, 1980; Newell, 1992) and soil ergosterol concentration has been reported to be associated with living fungal biomass in soil (West *et al.*, 1987) as opposed to the total (live and dead) hyphal length observed by the direct microscopic count method using calcofluor as stain. Therefore, live fungal biomass estimates based on soil ergosterol concentration theoretically should be less than the total fungal biomass estimates based on direct microscopic counts using calcofluor.

Estimates of total fungal hyphal length and fungal biomass by an independent laboratory using methods different than ours were outside of the range of values obtained by observers in our lab. Microbial Biomass Service uses the agar film technique which often gives higher estimates of fungal biomass than does the membrane filter method, although the two methods usually are well correlated (Bååth and Söderström, 1980). We also calculated fungal biomass for the sample using the hyphal length estimate of Microbial Biomass Service (316.3 m g soil$^{-1}$) and the formula cited in the methods section, arriving at a value of 0.87 mg g soil$^{-1}$, still outside the range of values obtained in our lab. The disparity may be due to methodology or observer variability but because there is presently no way to calibrate direct counting methods or biomass calculations, it is impossible to determine which estimate is most accurate. These results should be considered in light of the fact that both of our labs have experience in direct microscopic methods for estimation of fungal biomass. In any case, these findings indicate that great care should be exercised when comparing fungal biomass values obtained by one lab to those obtained by another.

The use of computerized image analysis systems has been proposed to eliminate observer variability from direct microscopic counts of fungi in soil (Morgan *et al.*, 1991). This method has the potential to eliminate observer error and fatigue-induced bias but has not yet been widely tested. However, this type of system requires an expensive, high-resolution camera and sophisticated, commercially-unavailable software capable of differentiating fungal from non-fungal filaments in a sample. Calibration of image analysis systems (determination of accuracy) for quantification of fungal hyphae in soil and lab to lab standardization remain a problem.

Coefficients of variation associated with direct microscopic counts of hyphae were generally from 2 to 2.5 times higher than those of ergosterol determinations. This is an important disparity between these methods when statistical comparisons of fungal presence in different treatments or ecosystems are to be made. Statistically significant disparities in fungal biomass based on the direct microscopic count method are more likely to be masked by method-associated variance than are those based on ergosterol concentration. Accuracy in measurements of fungi is also important when attempting to evaluate or quantify their role in ecosystem processes such as decomposition of organic residues or assessing soil C or N pools. Because fungi may often represent the dominant component of the total soil microbial community, sensitive and reliable estimates of their biomass is crucial to understanding the dynamics of ecosytem function.

In summary, the most significant source of error in the direct microscopic method of estimating fungal biomass in soil is observer subjectivity. A smaller source of error is due to variability of fungal content of sampling sites. The sampling error can probably be minimized by collecting many soil samples from the area that fungal biomass is to be estimated; however, error due to observer variability, especially between labs, may be more difficult to reduce. To minimize observer variability within our lab, we plan to use more extensive training including photomicrographs and a video monitor on our microscope to allow a number of observers to see and discuss a particular field of view simultaneously. In this way, we hope to reduce differences among observers in how they interpret what is seen under the microscope. The use of photomicrographs shared among labs may also prove to be a useful method for calibration among independent laboratories.

The high degree of subjectivity associated with direct microscopic observation and difficulties in standardization and calibration are drawbacks to this method. On the other hand, the uncertainty of converting soil ergosterol concentrations to fungal biomass values is also a problem. The advantages of these methods are that microscopic counting enables

direct observation of fungal hyphae in soil and soil ergosterol determinations involve less subjectivity and variability. While we will continue to use direct microscopic counting as our primary method of estimating soil fungal biomass, the results of our study support the use of ergosterol as a measure of fungal biomass in soil and indicate that further work on the refinement of factors for converting soil ergosterol concentrations to fungal biomass estimates will be useful. In the meantime, we recommend the use of both direct microscopic counts of fungal hyphal lengths and soil ergosterol concentrations as a way to increase the reliability and accuracy of estimating fungal biomass in soil and aid in comparison of estimates from different laboratories.

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